Second quarterly progress report on the *in vitro* production of *Acacia mangium x auriculiformis* hybrid and *Acacia crassicarpa* clones from Sabah Softwoods Sdn. Bhd. carried out by the Plant Biotechnology Laboratory (ICSB/CIRAD-Forêt, Tawau) :

May-July 1998

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This *in vitro* production started at the end of January 1998 and different dates of *in vitro* introduction and multiplication subcultures were carried out during the first three-month period according to the different plant sources (see last 1st quaterly report from February to April 1998).

CALENDAR OF THE OPERATIONS CARRIED OUT :

1- *Acacia mangium x auriculiformis* hybrids :

1-1- *Acacia* hybrid clones from Ulu Kukut, Brumas and Silam :

The shoots were initially collected from young cuttings consisting of 16 superior tree clones selected by SSSB and currently maintained in their nursery in Brumas : clones no. 1 to 16 (except no. 12 which was not available) and clone 483/34, the clone numbers having been assigned by SSSB. They were introduced *in vitro* on January 29, 1998.

a) Previous culture cycles :

- May 15, 1998 : Second multiplication subculture, 2nd batch :

Seven weeks after a first multiplication subculture (March 27, 1998), 222 plantlets were transferred to fresh multiplication media from 79 initial explants (Multiplication rate/7 weeks = 2.81).

- June 5, 1998 : Third multiplication subculture, 1st batch :

Six weeks after a second multiplication subculture (April 23, 1998), 447 plantlets were transferred to fresh multiplication media from 120 initial explants (Multiplication rate/6 weeks = 3.72).

b) Current culture cycles :

- July 7, 1998 : Third multiplication subculture, 2nd batch :

Eight weeks after a second multiplication subculture (May 15, 1998), **576** plantlets were transferred to a fresh multiplication medium from 209 initial explants (Multiplication rate/7-8 weeks = 2.76).

- July 16, 1998 : Fourth multiplication subculture, 1st batch :

Six weeks after a third multiplication subculture (June 5, 1998), **771** plantlets were transferred to a fresh multiplication medium from 312 initial explants (Multiplication rate/6 weeks = 2.47).

1-2- *Acacia* hybrid clones from the SSSB 1993 clonal test :

The shoots were initially collected from stock plants (marcots) consisting of 15 superior clones selected by SSSB and maintained in their nursery in Brumas. The corresponding SSSB clone numbers are as follows : AA17F105, AA7D15, AA7F110, AM7C9, AM7C3, AA7D16, AA7D6, AA7F112, AA7F113, AA7D18, AA7F117, AA7D12, AA7D17, AA7F72 and AA7F45.

Two batches of *in vitro* introduction were necessary as we obtained quite high contamination rates in culture (March 6 and April 23, 1998, respectively).

a) Previous culture cycles :

- May 23, 1998 : Second multiplication subculture, 1st introduction :

Six weeks after a first multiplication subculture (April 9, 1998), 222 plantlets were transferred to fresh multiplication media.

- June 22, 1998 : First multiplication subculture, 2nd introduction :

Two months after *in vitro* introduction (April 23, 1998), 42 plantlets of 11 clones were transferred from the introduction medium to a multiplication medium.

b) Current culture cycles :

- July 13, 1998 : Third multiplication subculture, 1st introduction :

Six weeks after the second multiplication subculture (May 23, 1998), **613** plantlets were transferred to fresh multiplication media from 213 initial explants (Multiplication rate/6 weeks = 2.88).

- July 17, 1998 : Second multiplication subculture, 2nd introduction :

Five weeks after a first multiplication subculture (June 12, 1998), **56** plantlets were transferred to a fresh multiplication medium from 36 initial explants.

So far, 13 of the 15 initial clones from the SSSB 1993 clonal test were successfully introduced (clones no. AA17F105 and AA7F117 were lost).

2- *Acacia crassicarpa* :

After a first stage of data assessment, selection and identification of the 20 best individuals among the best families - in terms of volume growth and bole straightness more especially - of the SSSB 1995 progeny trial in Brumas that was performed in collaboration with the SSSB and PISP teams, shoots were directly collected from tree branches of the selected plus trees before *in vitro* introduction. At the same time, marcots from the selected plus trees were performed by the SSSB team for further clonal propagation by coppicing.

Current culture cycles :

- April 23, 1998 : First *in vitro* introduction :

Shoots from 11 plus tree clones were collected : clones no. CR1 to CR11. Those plus trees were identified as the best individuals from the five best families ranked among a total of 62 families tested. Single-node segments were excised from the collected shoots and introduced *in vitro* after a disinfection treatment. A mean number of 48 explants per clone were introduced (total = 523 explants).

After a second transfer onto fresh medium (June 12,1998) and then 3 months after introduction, the number of reactive explants was very low with only **8** responsive explants exhibiting slow-growing axillary shoots or buds whereas **76** nodes were still alive but not reactive so far : 2 shoots for CR1 ; 1 for CR3 ; 3 for CR5 ; 1 for CR6 and 1 for CR10 (percentage of infection/total number of introduced shoots = 84%).

- June 4, 1998 : Second *in vitro* introduction :

Shoots from 14 plus tree clones were collected : clones no. CR2, CR7 and CR9 to CR20 . A mean number of about 38 explants per clone were introduced (total = 526 explants).

To date, *i.e.* six weeks after introduction, the number of reactive explants was very low with only **9** responsive explants exhibiting growing axillary shoots among **107** alive nodes but not reactive so far : 1 shoot for CR7 ; 4 for CR9 ; 2 for CR10 ; 1 for CR12 and 1 for CR14 (percentage of infection/total number of introduced shoots = 78%).

- July 11, 1998 : Third *in vitro* introduction :

Shoots from 11 plus tree clones were collected. They originated from the rooted marcots recently transferred from the 1995 Progeny trial to the SSSB Research nursery : clones no. CR1 to CR11. A mean number of about 27 explants per clone were introduced (total = **292** explants).

The responsiveness of the explants collected from the marcots could be better than that of the explants collected directly from the tree branches as they consisted of young shoots isolated at a very active stage of development (as that is the case for some of the SSSB Acacia hybrid clones mentioned above).

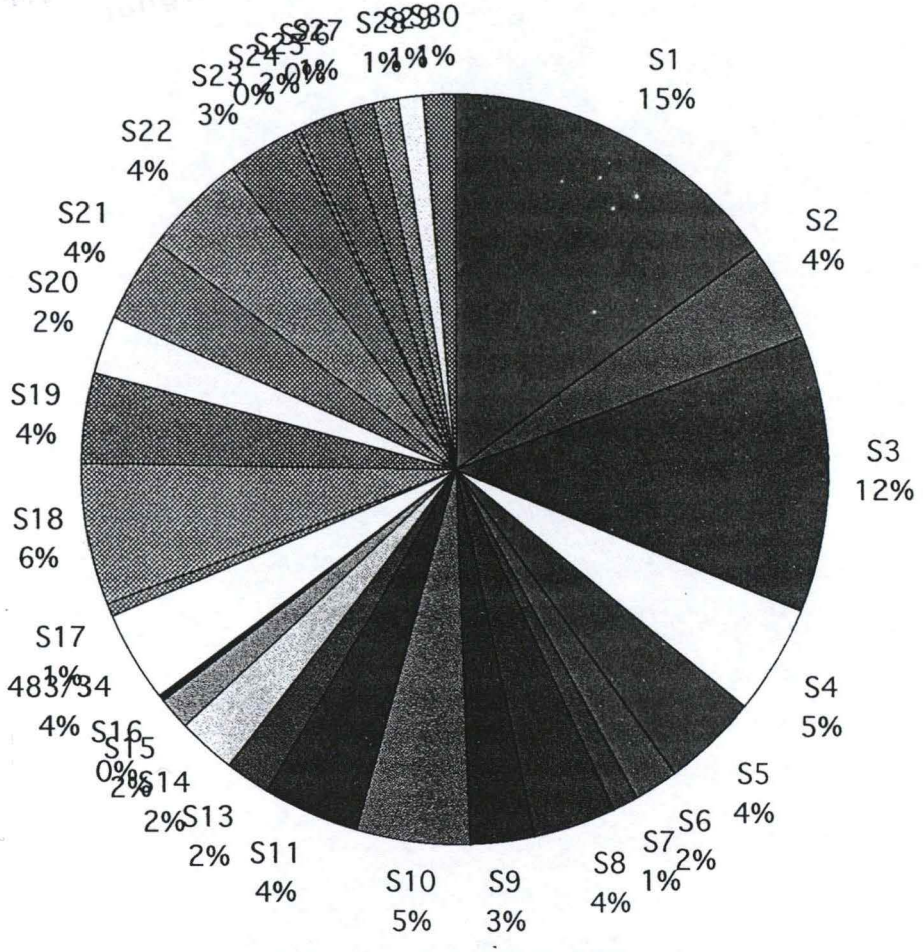
Conclusion :

At the end of July, 2016 Acacia hybrid and 492 Acacia crassicarpa plantlets are currently under multiplication (see the proportion of the different hybrid clones in the attached figure hereafter). However, most of the Acacia crassicarpa plantlets consist of non reactive nodes. The next transfer of all batches is planned for the end of September. According to our predictions, that are based on a current multiplication mean rate of 3 every 2 months and an average rooting percentage of 75% for all the *Acacia* species concerned (after two months of culture in the rooting medium), the actual number of plantlets seems to be satisfactory to deliver the 20,000 expected plantlets to SSSB on time (i.e. January and February 1999) if no major problems of infections or room temperature regulation occur by then.

SUMMARY TABLE : *IN VITRO* PRODUCTION OF *ACACIA* SELECTED CLONES FROM SSSB (May-July 1998)

Species	Origin	Batch of introduction	Date of introduction	Number of explants	Date of 2nd Multiplication Subculture	Number of explants	Date of 3rd Multiplication Subculture	Number of explants	Date of 4th Multiplication Subculture	Number of explants
***Acacia* hybrids**	16 clones from Ulu Kukut, Brumas & Silam	no. 1	-	-	-	-	June 5	312	**July 7**	**771**
		no. 2	-	-	May 15	209	**July 7**	**576**	-	-
"	13 clones from 1993 Clonal test	no. 1	-	-	May 23	213	**July 13**	**613**	-	-
		no. 2	-	-	July 17	56	-	-	-	-
Acacia crassicarpa	20 clones from 1995 Progeny trial	no. 1	**April 23**	**84**	-	-	-	-	-	-
		no. 2	**June 4**	**116**	-	-	-	-	-	-
		no. 3	**July 11**	**292**	-	-	-	-	-	-

Note : current culture cycles are marked in bold characters.


SSSB Acacia hybrid clones under in vitro multiplication on July 20, 1998
S1 15%
S2 4%
S3 12%
S4 5%
S5 4%
S6 2%
S7 1%
S8 4%
S9 3%
S10 5%
S11 4%
S13 2%
S14 2%
S15 0%
S16 4%
S17 1%
S18 6%
S19 4%
S20 2%
S21 4%
S22 4%
S23 3%